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(54) Title: A NOVEL METHOD TO PROTECT PLANTS FROM FUNGAL INFECTION

(57) Abstract

A method for protecting a crop against fungal diseases comprising applying to the crop or its locus a composition containing an effective amount of an aminobutyric acid or derivatives thereof.

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A NOVEL METHOD TO PROTECT PLANTS FROM FUNGAL INFECTION

INTRODUCTION

The present invention concerns a novel method to protect plants from pathogenic attack. The present invention more particularly concerns a method of applying selected compounds and compositions to a crop and its locus to induce local and systemic resistance of the crop to fungal diseases, wherein such action is referred to in this application as "induced plant defence" (hereinafter IPD).

BACKGROUND OF THE INVENTION

IPD in a crop is manifested by various defense mechanisms including the accumulation in the crop of soluble proteins referred to as pathogenesis-related (PR) proteins. Some PR proteins have been shown to be hydrolytic enzymes such as chitinases and B-1, 3 - gluconases, while others are shown to be peroxidases. Also accumulated are a group of these proteins having a molecular weight or about 10 to 20 kDaltons referred to as P14 proteins, which are without known biological function. All of these proteins are believed to participate in the defense system of a crop. Various isonicotinoyl-pyridinyl-hydrazine-derivatives and benzothiadiazole compounds have been described in the patent literature as immunizing healthy plants against fungal diseases (European Patent Applications 0 268 775, 0 288 976 and 0 313 512). The use of DL-ß-amino-butyric acid for the control of root rot of peas caused by Aphanomyces euteiches Drechs. has also been described (Papavizas, Plant Disease Reporter, Vol. 48, No. 7.pp. 537-541 (1964), Papavizas, Plant Disease Reporter, Vol. 51, No. 2, pp. 125-129 (1967).

The use of DL-α-aminobutyric acid at 0.03 M was described for the control of apple scab caused by Venturia

inaequalis (Kuc et al., 1959, Phytopathology 49:313-315). The use of DL-α-aminobutyric acid and DL-ß-aminobutyric acid was described as chemotherapeutants against the fungi Colletotrichum cucumerinum and Colletotrichum lagenarium on cucumber and Phytophthora infestans on tomato (Oort, A.J.P. and Van Andel, O.M., 1960, Mededel, Landbouwhogeschool Opzoekingssta. Staat Gent 25:981-992).

Various derivatives of DL-ß-aminobutyric acid and ß-aminocrotonic acid have been described in the patent literature as fungicides against Phytophthora infestans in tomato and Plasmopara viticola in grapes (DE-PS 1120802)

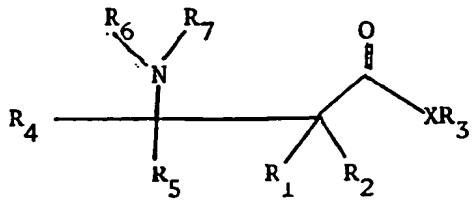
Recently, Cohen, Niderman, Mosinger and Fluhr (Plant Physiol.(1994) 104:58-66 reported that PR proteins are involved in IPD in tomatoes.

OBJECTIVES OF THE INVENTION

It is the objective of the present invention to provide a novel method to induce IPD. It is a further objective of the present invention to provide a novel method to induce IPD in selected crops.

SUMMARY OF THE INVENTION

We have found a novel method of protecting a crop against fungal diseases caused by fungi by applying to the crop or its locus a composition containing an effective amount of a compound of formula (I):



R_1 and R_2 are independently hydrogen, C_{1-8} alkyl, phenyl, and phenyl C_{1-4} alkyl

R_3 is hydrogen; C_{1-23} alkyl; carboxy C_{1-4} alkyl; phenyl C_{1-4} alkyl; wherein the phenyl moiety is unsubstituted or monosubstituted by halogen; or C_{2-23} alkoxy-carbonyl C_{1-4}alkyl;

R_4 and R_5 are independently hydrogen or C_{1-8} alkyl;

R_6 and R_7 are independently hydrogen; C_{1-8} alkyl C_{2-8} alkanoyl; phenyl C_{1-4} alkyl wherein the phenyl moiety is unsubstituted or monosubstituted by halogen; C_{2-8} alkoxy-carbonyl; $CONR_8$ wherein R_8 is is hydrogen, C_{1-8} alkyl, phenyl C_{1-4} alkyl; phenyl C_{2-4} alkyloxycarbonyl;

X is O, S or N, and salts thereof; and

the crop is selected from the group consisting of corn, cucumber, melon, broccoli, cauliflower, kohlrabi, potatoes, cabbage, sunflower, tobacco, grapes, cotton, maize, sorghum, pearl millet, rice, lettuce, hop, avocado, citrus, soybean, and onion.

DETAILED DESCRIPTION OF THE INVENTION

Alkyl as used herein refers to straight chains, branched and cyclic forms and preferably contain one to four carbon atoms.

R_1 and R_2 are preferably independently hydrogen, methyl or phenyl, more preferably R_1 is hydrogen or methyl and R_2 is hydrogen.

R_3 is preferably hydrogen.

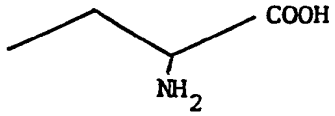
R_4 and R_5 are preferably independently hydrogen or C_{1-3} alkyl, more preferably R_4 is hydrogen or methyl and R_5 is hydrogen or C_{1-3} alkyl, more preferably R_4 is hydrogen or methyl and R_5 is hydrogen.

R_6 and R_7 are preferably independently hydrogen, C_{1-6} alkyl, benzyl optionally substituted by halogen, more preferably R_6 is hydrogen or methyl and R_7 is hydrogen.

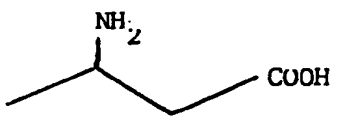
X is preferably oxygen.

Preferred compounds of the invention are the aminobutyric acids and the aminovaleric acids; and most preferred are the aminobutyric acids (Scheme I) and especially R - ß - aminobutyric acid.

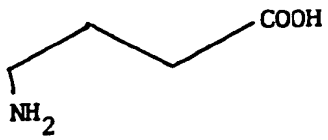
Scheme I



α-Aminobutyric acid
(referred to as "AABA")

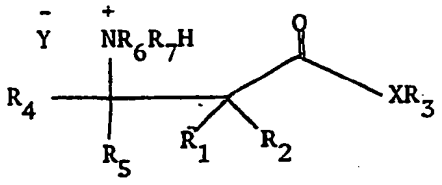


ß-Aminobutyric acid
(referred to as "BABA")



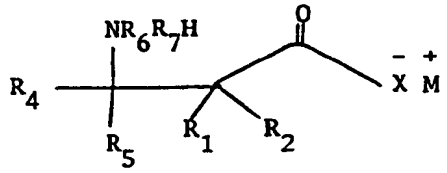
-Aminobutyric acid (referred to as "GABA")

Salt forms of the compound of formula (I) contemplated in this application include acid addition salts such as those obtained by the addition of HC_1, CF_3 CO_2H, toluene sulfonic acid, methane sulfonic acid and $(CO_2H)_2$



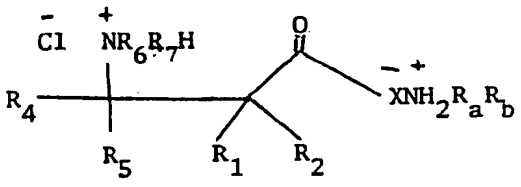
wherein Y is the residue of the acid;

alkali metal salts such as those obtained by treatment with NaOH, KOH or LiOH



wherein M is an alkali metal such as Na, K or Li; and

acid addition/amine salts such as those obtained by treatment with HCl and an amine such as diethylamine, propyl amine, benzylamine.



wherein R_a and R^b are substituents.

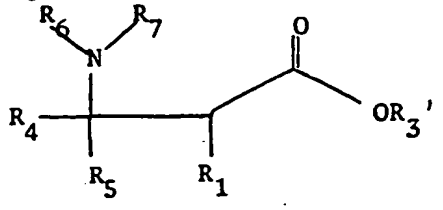
Preferred crops in which the method of the present invention is applicable are cucumbers, melon, broccoli, cauliflower, kohlrabi, potatoes, sunflower, tobacco, grapes, cotton, maize, sorghum, cabbage, pearl millet, rice, and hop. Most preferred are sunflower, grapes, cucumber, melon, broccoli, kohlrabi, cauliflower, potatoes, tobacco and maize.

The method of the present invention is not obvious in view of the prior art cited - especially that in regards to tomatoes. The reason is that the reported mechanism of IPD in tomatoes is via the formation of PR proteins. It has been found (Y. Cohen, unpublished results) that the mechanism in other plants is different. Thus it was found that the mechanism in, for example cucumbers and melons is via the accumulation of callose and lignin in the infected cells with no PR proteins involved. In addition, in tobacco it has been verified [Y. Cohen, Physiological and Molecular Plant Pathology (1994) 44: 273-288], that protection against fungal infections was not associated with either PR protein, callose, or lignin. Furthermore, the present method was effective against fungi already resistant to the major commercial chemical fungicides. Finally the present method was found to be effective even when applied post-infectionally, and therefore the method is a curative IPD.

Production methods

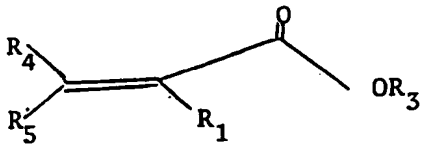
The novel compounds encompassed by the present application are structurally related to known compounds and can be easily prepared by either derivatising the known compounds or by modifying the procedures for preparing the known compound, as required. These procedures will be apparent to those skilled in the art The following procedures are illustrative.

Compounds of the formula (Ia)



(Ia)

wherein R_1 and R_{4-7} are as previously defined and R_3 represents hydrogen or C_{1-8} alkyl can be obtained from a compound of the formula (IIa).



(IIa)

To prepare compounds of formula (Ia) where R_6 is H and R_7 is as previously defined, the compound of formula (IIa) is reacted with NR_7H_2, wherein R_7 is as previously defined. Reactions of this type are described in the literature, e.g., by A. Zilkha and J. Rivlin, Joc 1957, 23, 94.

To prepare compounds of formula (Ia) where R_6 and R_7 are as previously defined but excluding hydrogen, the compound of formula (IIa) is reacted with NR_6R_7Li, wherein R_6 and R_7 are as previously defined but excluding hydrogen. Reactions of this type are described in the literature, e.g., by Davies et al., Tetrahedron: Asymmetry, Vol.2, No.3, pp. 183-186 (1991).

Compounds of the formula (IIa) are either known or obtainable from known compounds according to standard procedures.

As can be appreciated, in such cases where R_4 and R_5 do not represent the same substituent, the carbon atom to

which they attach is chiral. Procedures for preparing each enantiomer form are either specifically described in the literature, e.g., in EP 0 144 980 or in Davies, supra, or can be prepared according to analogous procedures.

The present method was found to be effective against a variety of diseases. Examples are late blight, downy mildew, blue mold, leaf spots, fusarium wilt, trunk rot, fruit brown rot, damping off, white rust, black shunk and phytophthora's root rots.

The compounds of this invention will typically be applied to crops or their locus before or after the onset or after the initial signs of fungal attack and may be applied to the foliar surfaces of the crop. The amount of the active ingredient to be employed will be sufficient to induce the systemic resistance of the crop to control the fungi and will vary depending on such factors as the species of fungi to be controlled, the type of treatment (for example, spraying dusting, seed treatment, soil drench), the condition of the crop, and the particular active ingredient used.

As an application to the crop or its locus, the compounds will be applied to the crops with a dosage rate of from 0.1 to 5 kg/ha, preferably from 0.2 to 2kg/ha. with application being repeated as necessary, typically at intervals of every one to three weeks.

Depending on circumstances, the compounds of this invention may be used in association with other pesticides, e.g., fungicides, insecticides, acaricides, herbicides, or plant growth regulating agents in order to enhance their activity or to widen their spectrum of activity.

The compounds of this invention are conveniently employed as fungicidal compositions in association with agriculturally acceptable carriers or diluents. Such compositions also form part of the present invention. They may contain, aside from a compound of formula (I) as active agent, other active agents, such as fungicides. They may be employed in either solid or liquid application forms e.g., in the form of a wettable powder, an emulsion concentrate, a water dispersible suspension concentrate ("flowable"), a dusting powder, a granulate, a delayed release form incorporating conventional carriers, diluents and/or adjuvants. Such compositions may be produced in conventional manner, e.g. by mixing the active ingredient with a carrier and other formulating ingredients.

Particular formulations to be applied in spraying forms such as water dispersible concentrates or wettable powders may contain surfactant such as wetting and dispersing agents, e.g., the condensation product of formaldehyde with naphthalene sulphonate, an alkyl-aryl-sulphonate, a lignin sulphonate, a fatty alkyl sulphate an ethoxylated alkylphenol and an ethoxylated fatty alcohol.

In general, the formulations include from 0.01 to 90% by weight of active agent, said active agent consisting either of at least one compound of formula (1) or mixture thereof with other active agents, such as fungicides. Concentrate forms of compositions generally contain between about 2 and 80%, preferably between about 5 and 70% by weight of active agent. Application forms of formulation may, for example, contain from 0.01% to 20% by weight, preferably from 0.01% to 5% by weight, of active agent.

Formulation Example I: Wettable powder

50 parts by weight of a compound of formula (I) are ground with 2 parts of lauryl sulphate, 3 parts sodium lignin the sulphonate and 45 parts of finely divided kaolininite until the mean particle size is below 5 microns. The resulting wettable powder so obtained is diluted with water before use to a concentration of between 0.01% to 5% active ingredient. The resulting spray liquor may be applied by foliar spray as well as by root drench application.

Formulation Example II: emulsion concentrate

25 parts by weight of a compound of formula I, 65 parts of xylene, 10 parts of the mixed reaction product of an alklphenol with xyleneoxide and calcium-dodeyl-benzene sulphonate are thoroughly mixed until a homogeneous solution is obtained. The resulting emulsion concentrate is diluted with water before use.

Formulation Example III: Granulate (for soil treatments)

Onto 94.5 parts by weight of quartz sand in a tumbler mixer is sprayed 0.5 parts by weight of a binder (non-ionic tenside) and is thoroughly mixed. 5 parts by weight of compound of the formula (I) in powdered form are then added and thoroughly mixed to obtain a granulate formulation with a particle size in the range of from about 0.3 to about 0.7 mm. The granulate may be applied by incorporation into the soil adjacent the plants to be tested.

Formulation Example IV: Seed or Tuber Dressing

25 parts by weight of compound of the formula (I), 15 parts of dialkylphenoxypoly (ethylenoxy) ethanol, 15 parts of fine silica,, 44 parts of fine kaolin, 0.5 parts of a colorant (e.g., crystal violet) and 0.5 parts of xantham gum are mixed and ground in a contraplex mill at approximately 10,000 rpm to an average particle size of below 20 microns.

The resulting formulation is applied to the seeds or tubers as an aqueous suspension in an apparatus suitable for that purpose. Where the compound of the formula (I) is liquid, it is first absorbed on the carriers, if desired with the air of a small amount of a volatile solvent such as acetone. The resulting powder is first allowed to dry if a solvent is used, then the other ingredients are added and the rest of the procedure is carried out.

Formulation Example V: Soil Drench Drip Irrigation

2 parts by weight of compound of the formula (I) are dissolved in 1,000 parts of water. The resulting formulation is applied to plants by drip irrigation .

As previously mentioned, the compounds of formula (I) are effective in activating or enhancing a crop's defense system against fungal diseases caused by fungi. Such activity can be demonstrated in using the general procedures of the following tests:

Test A: IPD in potato plants against Phytophthora infestans . Potato plants (cultivar Bintje) are grown from tubers in pots filled with sandy loam, peat and perlite mixed in equal volumes, in the greenhouse

(20-22°C). The plants are ready for testing when they have 6 or 7 compound leaves.

Tests are carried out with the metalaxyl-resistant isolated MR1 of Phytophthora infestans, as well as the MS2, MS3, MR2 and MR3 isolates (Kadish and Cohen, Phytopathology, 78:912-9155 (1988). The fungus is grown on potato tuber slices at 15°C in the dark. Fresh sporangia are harvested at 6 days after inoculating the slices into double distilled water (4°C) and their concentration adjusted to 10,000 sporangia/ml before used for challenge inoculations.

The compounds of this application are dissolved in water and sprayed on either the lower or upper leaf surfaces of the potato plants with a fine atomizer (about 10 ml per plant). The plants are left on the bench until the droplets dry and then are placed in a growth cabinet calibrated to 20°C and 14 hours of light per 10 day.

Challenge inoculation with P.infestans is carried out at time intervals ranging from 30 minutes to 12 days after treatment with the compounds, by spraying a sporangial suspension on the upper leaf surfaces (about 15 ml per plant). In one experiment, inoculum droplets (10 containing about 100 sporangia) are placed on the leaf surfaces, two droplets per leaflet, one on each side of the main vein. In another experiment the compounds are applied curatively, 24 hours after inoculation with P.infestans.

Inoculated plants are kept at 100% RH in the dark for 24 hours at 20 18°C and then returned to a growth cabinet maintained at 20° C with 12 hours light per day. Disease severity is monitored 4-8 days after inoculation by visually estimating the proportion or a leaf area covered by blight lesions.

Test B: IPD in tobacco plants against Peronospora tabacina . Tobacco plants (cultivars Ky-14 or Ky-16) are grown from seed in pots in the greenhouse. When reaching the 10-leaf stage or older the compounds of this application are injected into the stem of the plant. At 1-3 days before injection or at 1-10 days after injection plants are challenge-inoculated with conidia of the fungus Peronospora tabacina Adam which causes the blue mold disease. Conidia are harvested from previously infected tobacco plants. Inoculation is done with 10,000-100,000 conidia/ml, with approximately 50ml per plant. The procedure described above for inoculation, maintaining and scoring the disease are also applicable here.

While the invention will now be described in connection with certain preferred embodiments in the following examples, it will be understood that it is not intended to limit the invention to these particular embodiments. On the contrary it is intended to cover all alternatives, modifications and equivalents as may be included within the scope of the invention as defined by the appended claims. Thus, the following examples, which include preferred embodiments, will serve to illustrate the practice of this invention, it being understood that the particulars shown are by way of example and for purposes of illustrative discussion of preferred embodiments of the present invention only and are presented in the cause of providing what is believed to be the most useful and readily understood description of procedures, as well as of the principles and conceptual aspects of the invention.

EXAMPLES

EXAMPLE 1: N-(2-hydroxyethyl)-aminobutyric acid

A solution of 86g of crotonic acid (1 mole) and ethanolamine (1 mole) in pyridine (200ml) is refluxed for 2-3 hours and subsequently cooled. The resulting product is filtered and recrystallized to yield the title compound having m.p. 178-180° C (compound1.1, Table 1).
Following analogous procedure, the compounds 1.2-1.7, 1.10, 1.11, and 1.13-1.15 set forth in Table 1 are obtained.

EXAMPLE 2: 3-aminohexanoic acid

A mixture of 2 hexenoic acid (7.0g, 0.06 mol) and concentrated aqueous ammonium hydroxide (70ml) is heated for 24 hours in an autoclave at 150°C. The cooled mixture is treated with carbon black and filtered. After evaporation of the solvent the crude product is recrystallized from ethanol to give the title compound m.p. 203°C (compound 1.21, Table 1).
Following an analogous procedure, the compounds 1.8, 1.9, 1.12 and 1.18-1.20 of Table 1 are obtained.

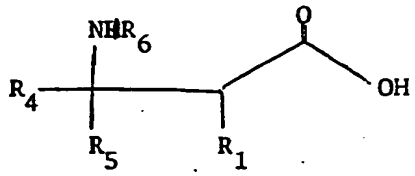
EXAMPLE 3: N-benzoyl-3-aminobutyric acid

To a cooled solution of 3 - aminobutyric acid (13g) in 2M NaOH (130ml) is added benzoyl chloride (19.7g) over the course of two hours. The mixture is allowed to warm to room temperature. Washing with diethyl ether, acidifying of the aqueous phase with 20% HCl, extraction with diethyl ether, drying over $MgSO_4$, evaporation of the solvent and recrystallization in ether/hexane gives the title compound m.p. 150-152°C (compound 1.16, Table 1).

Following an analogous procedure, compound 1.17 of Table 1 is obtained.

TABLE 1

COMPOUNDS PREPARED OF THE FORMULA:



Cpd	R_1	R_4	R_5	R_6	m.p. (°C)
1.1	H	CH_3	H	hydroxyethyl	178-180
1.2	H	CH_3	H	isopropyl	167-169
1.3	H	CH_3	H	benzyl	178-180
1.4	H	CH_3	H	cyclohexyl	161-163
1.5	H	CH_3	H	n-hexyl	151-153
1.6	H	CH_3	H	p-chlorobenzyl	152-154
1.7	H	CH_3	H	benzyl	180-182
1.8	H	ethyl	H	H	128-130
1.9	H	CH_3	CH_3	H	216
1.10	CH_3	H	H	benzyl	148
1.11	H	CH_3	H	phenyl-ethyl	164
1.20	H	phenyl	H	H	220-221
1.13	H	CH_3	H	n-octyl	150
1.14	H	CH_3	H	n-decyl	148
1.15	H	ethyl	H	benzyl	157-160
1.16	H	CH_3	H	benzoyl	150-152
1.17	H	CH_3	H	benzyloxycarbonyl	128-130
1.18	H	ethyl	H	H	178-180
1.19	H	CH_3	H	H	209-210
1.20	H	CH_3	H	CH_3	86-87
1.21	H	propyl	H	H	203

*(R) - enantiomer

**monohydrate

EXAMPLE 4: N-benzyloxycarbonyl - 3 - aminobutyric acid (4-chlorophenyl)-1- ethylamide

Z-protected ß - aminobutyric acid (0.02ml), (4-chlorophenyl)-1- ethylamine and 1.1 equivalents of DCC (dicyclohexyl carbonimide) are stirred in ethyl acetate at room temperature for 16 hours. The precipitate is filtered, the filtrate evaporated and chromatographed on silica gel (hexane/ethyl acetate 1:1) to give the title compound as a mixture of diastereomers m.p. 168-178°C.

EXAMPLE 5: ß-aminobutyric acid hydrochloride

5.15 g ß-aminobutyric acid (50 mol) are dissolved in 650ml methanol. After addition of 5.5ml concentrated HCl the solution is evaporated. The residue is triturated in diethyl ether, decanted and dried. A colorless oil is isolated. Microanalysis: C,34.4; H, 7.2; N, 10.0; Cl, 25.4.

EXAMPLE 6: ß-aminobutyric acid sodium salt

2.06g ß-aminobutyric acid (20mol) are dissolved in 100ml of a mixture of water:methanol (1:1). One equivalent NaOH in 10 ml water is added. The solution is evaporated and the resulting amorphous solid is dried. Microanalysis: C, 37.4; H, 6.7; N, 10.9.

EXAMPLE 7: ß-aminobutyric acid diethylammonium chloride

1.4 g ß-aminobutyric acid (10 mol) are dissolved in 100 ml of methanol. Diethylamine (0.9 g, 12.3 mol) is added and the residue is evaporated. The oily residue is

washed with ether, decanted and dried to afford an amorphous material. H-NMR (CD_3 OD, 200MHz) 1.29 (m, 9H, 3 CH_3; 2.25-2.45 (m, 2H, CH_2); 3.14 (p, 4H. CH_2 CH_3); 3.34-3.58 (m, 1H,CH)

EXAMPLE 8: Protection of tomato plants against fusarium wilt

Tomato plants were grown in sterile soil in the greenhouse. When they reached the 4-leaf stage they were treated with compound of the formula (I) solution by a soil drench. Four days later the plants were uprooted, washed with water and their root system immersed for two minutes in conidial suspension (10^7 conidia/ml) of the fungus Fusarium oxysporum f.sp. lycopersici. Plants were then transplanted (without washing) in pots filled with sterile soil.Twelve days later all challenged - control plants wilted of the disease whereas none of the challenged treated plants wilted. Growth of the latter plants was similar to that of control, unchallenged-uninoculated plants. Results are shown in Table 2.

EXAMPLE 9:

Following the method of Example 8, a similar experiment was run using lower concentration of BABA. The results are shown in Table 3.

TABLE 2

Protection of tomato plants (cv. Rehovot - 13) against fusarium wilt caused by Fusarium oxysporum f.sp. lycopersici by aminobutyric acids (soil drench)

Compound	Percent of plants	
	Healthy	Wilted
None	0	100
AABA	34	66
BABA	100	0
GABA	7	93

Plants were soil-drenched with 2000 ppm of the compound and inoculated 4 days later; rating was taken 12 days after inoculation.

TABLE 3

Protection of tomato plants (cv. Rehovot - 13) against fusarium wilt caused by Fusarium oxysporum f.sp. lycopersici by lower concentrations of BABA (soil drench)

Concentration (ppm)	Percent Wilted Plants
0	100
250	53
500	43
1000	0

EXAMPLE 10:

Following the above described methods, the effect of aminobutyric acids on downy mildew in sunflower were studied. The results appear in Table 4, which shows the marked activity of "BABA" in percent protection.

EXAMPLE 11:

Following the above described methods, the effect of aminobutyric acids on Plasimopara viticola in grape plants were studied. The results which appear in Table 5, show the good protection given by "BABA".

EXAMPLE 12:

Following the above described methods, the effect of aminobutyric acids on downey mildew in cucumber and melon plants were studied. The results, which appear in Table 6, show the good protection given by "BABA".

EXAMPLES 13 - 15:

Following the above described methods, the effect of R-BABA and S-BABA against P. parasitica and A. bassicola in broccoli, Kohlrabi, and cauliflower were studied; and the results, which appear in Tables 7 - 9, respectively, show the good protection given by R-BABA.

TABLE 4:

The effect of aminobutyric acids on systemic downey mildew of sunflowers (cv. D.I.-3) caused by Plasmopara halstedii .

Compound	Method of Application	Conc. mg/l	Percent Protection
AABA	spray	2000	10
BABA	spray	2000	100
GABA	spray	2000	0
		mg/Plant	
AABA	soil drench	5	0
BABA	soil drench	5	100
GABA	soil drench	5	0
AABA	root uptake	2	0
BABA	root uptake	2	100
GABA	root uptake	2	0

In all experiments inoculation with the fungus was done either 1 day before treatment (curative) or 2 days after treatment.

TABLE 5:

The effect of aminobutyric acids on downy mildew caused by Plasmopara viticola in grape plants (vc. Sauvignon blanc or Cabarnet sauvignon)

Conc. ppm	Application mode	%protection with AABA	BABA	GABA
10mg per plant	soil drench	0	60	0
100	spraying }	0	30	0
200	intact }	0	60	0
500	plants }	15	90	5
1000	in pots }	20	95	5
10		0	90	0
50	floating }	5	95	0
100	leaf discs }	10	100	5

Disease rating was taken 9 days after inoculation. BABA has also curative effect as follows: When applied to inoculated leaf discs at 0, 1, 2 and 3 days after inoculation percent protection was 100, 86, 50 and 30 percent, respectively.

TABLE 6:

The effect of aminobutyric acids on downy mildew caused by Pseudoperonospora cubensis in cucumber and melon plants (foliar spray) or leaf discs (floating)

Plant & cultivar	Conc. ppm	Percent protection by whole plants AABA	BABA	GABA
cucumber (Dlila)	250	0	0	0
	500	0	38	0
	1000	0	65	0
	2000	5	87	0
melon (Galia)	250	0	30	0
	500	0	38	0
	1000	0	86	0
	2000	0	92	0
			leaf discs	
cucumber (Dlila)	6	0	94	0
	12	0	97	0
	25	0	100	20
	50	0	100	60
melon (Ananas)	6	0	19	0
	12	0	25	0
	25	0	70	0
	50	37	85	30

Disease rating was taken 7 days after inoculation.

TABLE 7: (Broccoli)

Protection of Broccoli (cv. Shugon) against Peronospora parasitica and Alternaria brassicicola with amino butyric acids applied as a foliar spray.

Compound	Conc. ppm	percent protection	
		P.parasitica	A.brassicicola
R-BABA	{125	33	0
	{250	50	20
	{500	95	60
	{1000	100	85
	{2000	100	90
S-BABA	{500	0	not tested}
	{1000	0	}
	{2000	0	}

TABLE 8: (Kohlrabi)

Protection of Kohlrabi (cv. White Wien) against Peronospora parasitica and Alternaria brassicicola with amino butyric acids applied as a foliar spray.

Compound	Conc.ppm	percent protection	
		P.parasitica	A.brassicicola
	{125	33	0
	{250	50	20
R-BABA	{500	95	60
	{1000	100	85
	{2000	100	90
S-BABA	{500	0	not tested}
	{1000	0	}
	{2000	0	}

TABLE 9: (Cauliflower)

Protection of Cauliflower (cv. Nurit) against Peronospora parasitica and Alternaria brassicicola with amino butyric acids applied as a foliar spray.

Compound	Conc.ppm	percent protection P.parasitica	A.brassicicola
R-BABA	{125	33	0
	{250	50	20
	{500	95	60
	{1000	100	85
	{2000	100	90
S-BABA	{500	0	not tested}
	{1000	0	}
	{2000	0	}

EXAMPLE 16:

Following the above described methods, the effect of a 25% formulated DL BABA in potatoes was studied. The results are shown in Table 10.

EXAMPLE 17:

Following the above described methods, the markedly good effect of DL - BABA against the control of late blight in potatoes (Bintje in growth chambers was studied. The results are shown in Table 11

EXAMPLE 18:

Following the method of Example 16 but running field trials in both Alpha and Bintje cultivars, the results are shown in Table 12.

EXAMPLES 19-20:

Resistance to Peronospora tabacina induced in tobacco plants was studied as outlined by Y. Cohen (Physiological and Molecular Plant Pathology (1994) 44:273-88) where the active ingredients were applied as a stem injection. Results for the stem injection are shown in Table 13.

EXAMPLE 21:

Following the method of Examples 19-20, the effect of a soil drench with DL- BABA (3mg per plant) on blue mold development in tobacco cv. Ky 16 showed an 80 percent control of the disease some 20 days after challenge inoculations.

TABLE 10

EFFECT OF 25% WP FORMULATED DL-BABA IN POTATOES

ppm DL-BABA	Percent Protection
Control	-
31	0
62	7
125	67
250	67
500	81
1,000	91
2,000	97

TABLE 11

Effect of aminobutyric acids against late blight in potato crops in growth chamber

Control	2	0
	4	80
	6	95
	8	98
	10	98
GABA	2	0
	4	60
	6	85
	8	90
	10	98
	2	0
	4	45
DL-AABA	6	70
	8	80
	10	98
DL-BABA	2	-
	4	0
	6	10
	8	10
	10	10

TABLE 12:

Percentage control of late blight epidemics induced by Phytopthora infestans (isolate MR1) in potato crops treated with BABA (25 WP) in three independent field experiments.

Experiment and cultivar	dose Kg a.i. per ha	Interval between sprays, days 7	10	14
Autumn Alpha	0	-	-	0
	0.2	-	-	24.6
	0.4	-	-	52.6
	0.8	-	-	55.1
Winter Bintje	0	0	0	0
	0.2	55.0	42.1	38.4
	0.4	57.4	52.5	47.1
	0.8	62.6	62.6	58.0
Spring Alpha	0	0	-	-
	0.57	38.0	-	-
	1.15	77.7	-	-
	2.30	75.0	-	-
Spring Bintje	0	0	-	-
	0.57	35.2	-	-
	1.15	64.5	-	-
	2.30	76.0	-	-

TABLE 13:

Resistance to Peronospora tabacina in tobacco plants by aminobutyric acids via stem injection

Compound	Stem Injection [c] Disease Severity (mean ± SD)
Water	2.0 ± 0a
DL- AABA	1.3 ± 0.2b
DL-BABA	0.7 ± 0.2c
R-BABA	0.07 ± 0.09d
GABA	2.0 ± 0a
SA[a]	1.5 ± 0b
INA[b]	0.5 ± 0c

[a] Sodium salicylate

[b] 2,6 dichloro-iso-nicotinic acid

[c] The letters refer to statistics.

EXAMPLE 22:

Sunflower plants were protected against downy mildew caused by Plasmopora halstedii by treating the seeds with BABA. Thus, the seeds were soaked for 24 hours in solution containing 10mg BABA per ml, and then sown in pots in the greenhouse.Two weeks later the developed plants were inoculated with Plasmopora halstedii . The progress of the disease was assessed after seven more days with the following results: While the control had 100 percent of the plants remained infected; the treated plants had only a 2 percent rate of infection.

EXAMPLE 23:

Maize (Line 3376) seeds were allowed to sprout in water for 5 days. They were then dipped in a BABA solution for one day. The sprouted seeds were washed and placed in contact with Fusarium moniliforme for one day and then planted in pots. After two weeks the progress of the disease was as follows:

BABA (ppm)	0	125	250	500	2,000
Percent plants infected.	70	80	40	0	0

EXAMPLE: 24

The activity of DL-α-aminobutyric and DL-ß-aminobutyric acids against late blight (Phytophthora infestans) on various potato cultivars in growth chambers was studied. Six week old plants in pots were sprayed with the compounds and inoculated two days later. Disease records taken 7 days post-inoculation are listed in Table 14.

TABLE 14

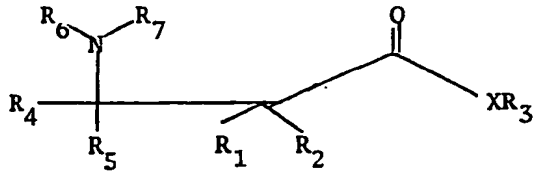
Activity of DL-α-aminobutyric and DL-ß-aminobutyric acids against late blight (Phytophthora infestans) on various potato cultivars in growth chambers.

Compound and conc.ppm	% protection			
	Nicola	Agria	Clauster	Spunta
DL-α 500	-	-	92	-
1000	-	-	97*	-
2000	71	86	97*	65
DL-ß 500	-	-	33	-
1000	-	-	73	-
2000	57	97	91	94

* = Some phytotoxicity.

WHAT IS CLAIMED IS:

1. A method for protecting a crop against fungal diseases caused by fungi comprising applying to the crop or its locus a composition containing an effective amount of a compound of formula (I):



wherein:

(a) R_1 and R_2 are independently hydrogen, C_{1-8} alkyl, phenyl, and phenyl C_{1-4} alkyl;

(b) R_3 is hydrogen; C_{1-23} alkyl; carboxy-C_{1-4} alkyl; phenyl C_{1-4} alkyl; wherein the phenyl moiety is unsubstituted or monosubstituted by halogen; or C_{2-23} alkoxycarbonyl C_{1-4} alkyl;

(c) R_4 and R_5 are independently hydrogen or C_{1-8} alkyl;

(d) R_6 and R_7 are independently hydrogen; C_{1-8} alkyl; C_{2-8} alkanoyl; phenyl C1-4 alkyl wherein the phenyl moiety is unsubstituted or monosubstituted halogen; C2-8 alkoxycarbonyl; CONR8 wherein R8 is hydrogen C1-8 alkyl, phenyl phenyl C1-4 alkyl; phenyl C2-4 alkyloxycarbonyl;

(e) X is O, S or N, and salts thereof; and the crop is selected from the group consisting of corn, cucumbers, melons, broccoli, cauliflower, kohlrabi, sunflower, potatoes, tobacco, grapes, cotton, cabbage, maize, sorghum, pearl millet, rice, lettuce, hop, avocado, citrus, soybean and onions in an amount sufficient to induce local and systemic resistance of the crop to control the fungal disease.

2. A method according to claim 1, wherein R_1 and R_2 are independently hydrogen, methyl or phenyl, R_3, is hydrogen, R_4 and R_5 are independently hydrogen or C_{1-3} alkyl, R_6 and R_7 are independently hydrogen, C_{1-6} alkyl or benzyl optionally substituted by halogen and X is oxygen.

3. A method according to claim 2, wherein R_1 is hydrogen or methyl, R_2 is hydrogen, R_4 is hydrogen or methyl, R_5 is hydrogen, R_6 is hydrogen or methyl and R_7 is hydrogen.

4. A method according to claim 1. wherein the compound of formula (I) is R ß-aminobutyric acid or ß-aminovaleric acid.

5. A method according to any of Claims 1 to 4 wherein the crop is selected from the group consisting of cucumbers, melon, broccoli, cauliflower, kohlrabi, potatoes, sunflower, tobacco, grapes, cotton, maize, sorghum, cabbage, pearl millet, rice and hop.

6. A method according to any of Claims 1 to 4 wherein the crop is selected from the group consisting of sunflower, grapes, cucumber, melon, broccoli, kohlrabi, cauliflower, potatoes, tobacco and maize.

7. A method according to any of Claims 1 to 6 wherein the compound is applied to the leaves or stems of the plant.

8. A method according to any of Claims 1 to 6 wherein the compound is applied to the roots of the plant.

9. A method according to any of Claims 1 to 6 wherein the compound is applied to the soil.

10. A method according to any of Claims 1 to 6 wherein the compound is applied to the seeds, tubers, or bulbs of the plant.

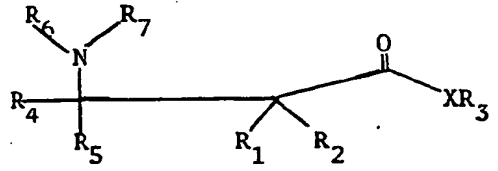
11. A method according to any of Claims 1 to 10 wherein the compound is applied pre-emergence.

12. A method according to any of Claims 1 to 10 wherein the compound is applied post-emergence.

13. A method according to any of Claims 1 to 12 wherein the compound is applied to the crop at a dosage rate of from 0.1 to 5 kg/ha.

14. A method according to any of Claims 1 to 12 wherein the compound is applied to the crop a dosage rate of 0.2 to 2 kg/ha.

15. A method for protecting tomatoes against Fusarium oxysporum of Sp. Lycopersici comprising applying to the tomato plant or its locus an effective amount of the compound of formula I:



wherein:

(a) R_1 and R_2 are independently hydrogen, C_{1-8} alkyl, phenyl, and phenyl C_{1-4} alkyl;

(b) R_3 is hydrogen; C_{1-23} alkyl; carboxy-C_{1-4} alkyl; phenyl C_{1-4} alkyl; wherein the phenyl moiety is unsubstituted or monosubstituted by halogen; or C_{2-23} alkoxycarbonyl C_{1-4} alkyl;

(c) R_4 and R_5 are independently hydrogen or C_{1-8} alkyl;

(d) R_6 and R_7 are independently hydrogen; C_{1-8} alkyl; C_{2-8} alkanoyl; phenyl C_{1-4} alkyl wherein the phenyl moiety is unsubstituted or monosubstituted halogen; C_{2-8} alkoxycarbonyl; $CONR_8$ wherein R_8 is hydrogen, C_{1-8} alkyl, phenyl C_{1-4} alkyl; phenyl C_{2-4} alkyloxycarbonyl; and

(e) X is O, S or N, and salts thereof.

16. A method according to claim 15, wherein R_1 and R_2 are independently hydrogen, methyl or phenyl, R_3 is hydrogen, R_4 and R_5 are independently hydrogen or C_{1-3} alkyl, R_6 and R_5 are independently hydrogen, C1-6 alkyl or benzyl optionally substituted by halogen and X is oxygen.

17. A method according to claim 15, wherein R_1 is hydrogen or methyl, R_2 is hydrogen, R_4 is hydrogen or methyl, R_5 is hydrogen, R_6 is hydrogen or methyl and R_7 is hydrogen.

18. A method according to Claim 15, wherein the compound of formula (I) is R-ß-aminobutyric acid or ß-aminovaleric acid.

19. A method according to any of Claims 15 to 18 wherein the compound is applied to the leaves or stems of the plant.

20. A method according to any of Claims 15 to 18 wherein the compound is applied to the roots of the plant.

21. A method according to any of Claims 15 to 18 wherein the compound is applied to the soil.

22. A method according to any of Claims 15 to 18 wherein the compound is applied to the seeds, tubers, or bulbs of the plant.

23. A method according to any of Claims 15 to 21 wherein the compound is applied post-emergence.

24. A method for protecting potatoes against fungal diseases selected from the group consisting of late blight, early blight and leaf spots, applying to potato plants or its locus a composition containing an effective amount of DL-α-aminobutyric acid, D-α-aminobutyric acid, L-aminobutyric acid, or mixtures thereof.

25. A method according to Claim 24 wherein the compound is applied to the leaves or stems of the plant.

26. A method according to Claim 24 wherein the compound is applied to the roots of the plant.

27. A method according to Claim 24 wherein the compound is applied to the soil.

28. A method according to Claim 24 wherein the compound is applied to the seeds, tubers, or bulbs of the plant.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/14108

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A01N 37/12, 37/18, 37/44, 47/10
US CL :514/478, 483, 485, 487, 488, 538, 551, 561, 562, 564, 619, 620
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/478, 483, 485, 487, 488, 538, 551, 561, 562, 564, 619, 620

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 3,899,585 (MISATO et. al.) 12 August 1975, see entire document.	1-28
Y	US, A, 3,991,208 (Dudzinski et. al.) 09 November 1976, see entire document.	1-28
Y	US, A, 4,481,219 (Watkinson) 06 November 1984, see entire document.	1-28
Y	US, A, 5,096,700 (Seibel et. al.) 17 March 1992, see entire document.	1-28
Y	GB, A, 1,048,507 (Harnack et. al.) 16 November 1965, see entire document.	1-28

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
08 MARCH 1995	20 MAR 1995
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